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A study on diagnostic performance of different immunohematological diagnostic tests in assessing the prevalence of ABO Hemolytic Disease of Newborn in the antenatal O group mothers and their neonatal outcome in a tertiary care hospital in Northern India

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ABSTRACT

Background: Hemolytic disease of the newborn (HDN) results in the decreased lifespan of the red cells. HDN related to ABO incompatibility is mostly unnoticed because routine screening is not being done. This study was done to assess the prevalence of ABO-HDN and to compare different immunohematological tests. Methods-In this study 213 O group mothers and the 122 ABO-incompatible newborns born to them were included. Quantifying the maternal IgG anti-A/anti-B antibody titer was done by Conventional Tube Technique (CTT) using Dithiothreitol (DTT) pretreated maternal serum. Hemolysin test was performed on the mothers having titer > 256. These cases were followed up and, after delivery, were monitored for ABO HDN, along with direct antiglobulin testing and elution studies. The prevalence of ABO-HDN was calculated, and the different diagnostic parameters of the tests were calculated. Results- The prevalence of ABO-HDN in our population was estimated to be 1.7%, 6.1% & 10.6% in our population, O group mothers, and O group mothers with ABOincompatible newborns, respectively. Maternal titer≥ 512 strongly correlated with ABOHDN. DAT positivity is a good predictor of ABO-HDN, especially using sensitive techniques. Maternal IgG titers have the highest sensitivity & Negative Predictive Value, while DAT has the highest specificity & Positive Predictive Value. Conclusion – Maternal ABO antibody titration may be advocated in the centers to identify high-risk groups. It can advocate institutional delivery and dedicated follow-up of newborns with ABO-HDN. Blood grouping & DAT may be performed in all newborns born to O blood group to identify high-risk cases.

1. Introduction

Hemolytic disease of the newborn (HDN) is a condition that results in decreased lifespan of the red cells in newborns due to trans-placental transfer of IgG antibodies [1,2]. In clinical practices and literature, the important primary type of HDN described is Rh D HDN [3]. ABO-HDN is considered less severe because of the weak fetal expression of A/B antigens on fetal red cells, wider expression of ABO antigens not confined to red cells, and less hemolytic potential of the IgG2 subclass of antibodies [1]. However, in the recent past, the spectrum of HDN had a shifting paradigm due to the widespread practice of normal postpartum prophylactic Rh immunoglobulin to D-negative mothers. [3] ABO incompatibility is now the most significant cause of HDN in the Western world [2]. ABO- HDN predominantly occurs in offsprings of O blood group mothers because IgG Anti-A/B are more in the O group than A or B groups. The clinical presentation is usually mild. Anemia is rare in ABO-HDN. A minor degree of red cell destruction is clinically pronounced as neonatal hyperbilirubinemia, which may lead to kernicterus and developmental disabilities [2]. Some studies have also postulated that the maternal antibodies directed against A and B antigens on red cell membranes lead to early abortion [4]. In this backdrop, the ABO-HDN spectrum is critical because ABO incompatibility or hemolysis in the fetus cannot be prevented and the clinical severity may not be severe enough for institutional check-ups and intervention. Hence, the

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reported cases of ABO-HDN form only the 'tip of the iceberg,' and proper antenatal screening is necessitated, especially in low resource countries. Many immunohematological tests have been utilized to predict the severity of HDN, like maternal anti-A/B titer, Direct Antiglobulin Test (DAT), or hemolysin test, for screening purposes.

This aim of this study was to analyze the diagnostic performance of the different immunohematological tests for screening of O group women with fetomaternal ABO incompatibility and their neonatal outcome. Furthermore, the prevalence of the disease was also studied in the study population that attended our institution.

2. Materials and methods

This prospective observational study was conducted in a tertiary care centre in North India over one year. Ethical approval was obtained from the institute's ethics committee prior to the study. Informed consent was also obtained from the study subjects. The study included all antenatal mothers with O blood group who delivered their babies at the institution. The donor population characteristics of the specific region were analysed and the risk of an event where an O group woman would give birth to a child who has a phenotype other than O was calculated. Based on previous studies, it was estimated that 20% of the group O study population has IgG anti-A/anti-B titer≥ 512 [5]. The maternal ABO-Rh group and anti-A/B antibodies titration was done in all the participant antenatal mothers in the last trimester of pregnancy. Antibody screening was done in all mothers. Hemolysin test was performed in the mothers with antibody titer≥ 256. The newborns born to the participants were followed up till the duration of hospital stay and, after delivery, were monitored for ABO-HDN. The ABO-Rh group of neonates was done, and neonates with non-O group were followed up. Direct antiglobulin test (DAT) and elution studies were done in all cases. Blood grouping and Anti A/Anti B titration was done by Conventional Tube Technique (CTT). For IgG antibody titration, Dithiothreitol (DTT) treatment was done. DAT was done by both CTT and Column Agglutination Technique (CAT) using commercially available poly-specific AHG cassettes [Ortho Clinical Diagnostics, Raritan, New Jersey USA]. Heat elution was performed per the department's Standard Operating Procedure (SOP). ABO-HDN was defined based on the following criteria [6].

i) High maternal IgG antibody titers.

ii) Positive DAT.

iii) Laboratory & clinical evidence of hemolysis, like anemia & hyperbilirubinemia within 24 h of life.

iv) Negative DAT with clinical evidence of hemolytic disease of the newborn.

The cases of ABO-HDN were graded into three categories as per the classification given by Choudhuri et al., described in Table 1 [7].

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSSV.23.0 for Windows). Descriptive variables were expressed as frequencies and percentages. Continuous variables were expressed as means ± standard deviation or as medians with total and interquartile ranges and compared using unpaired t-test, Mann-Whitney U-test, and Wilcoxon rank sum test depending upon the distribution of parameters. The sensitivity and specificity of ABO titer values and other parameters for predicting ABO-HDN were analysed by a receiver operating characteristic (ROC) curve, and the Yuden index was calculated.

Table 1
Classification of ABO-HDN.

Grade of ABO-HDN	Definition
Mild	Positive DAT alongside some evidence of hemolysis but requiring no intervention.
Moderate	Cases with evidence of hemolysis and intervention was limited to phototherapy
Severe	When the baby was hydropic or intrauterine/ exchange or top up transfusion was required.

The Spearman Rank Correlation was used to calculate the strength of association between maternal titres and total serum bilirubin. Arithmetical methods based on Hardy Weinberg equilibrium were used to determine the population prevalence of different ABO genes. The prevalence of high-titer mothers in this study population and ABO gene frequencies were used to calculate the likelihood of an event where an ABO-incompatible newborn at risk of developing ABO-HDN will be born to an O group mother.

3. Results

3.1. Estimating the Risk of ABO-HDN in the study population

Blood group data from blood donors in the specific region was analyzed from a previous study by Kaur et al. The frequency of different ABO blood groups reported in this study were 23.4, 35.6, 29.5, and 11.4% for A, B, O, and AB, respectively [8]. It was estimated that 14.36% of deliveries in this part of India would result in an event where an O group woman will give birth to a child who has a phenotype other than O. In this study,18% of group O mothers had IgG anti-A/B≥ 512. Based on similar studies conducted in this region, it was estimated that 2.86% of deliveries in the study population were at risk of ABO-HDN requiring intervention [5].

3.2. Prevalence of ABO-HDN in the study population

A total of 213 group O antenatal mothers were included in the study out of 732 antenatal mothers who delivered in the hospital during that period. Of the 213 group O mothers, 57.2% mothers delivered ABO-incompatible newborns. The total number of ABO-HDN cases reported in the study population was 13. The disease prevalence among antenatal mothers in the institution (n = 732) was 1.77%, while the same in O group mothers (n = 213) was 6.1% and the same in O group mothers with ABO incompatible newborns(n = 122) was 10.6%. Among 213 participants, 97.2% (207/213) of pregnant women were O Rh D positive, and 2.8% (6/213) were O Rh D negative. 45.9% (n = 56) of newborns had A blood group while 54.09% (n = 66) of newborns had B blood group. 92.3% (12/13) of ABO-HDN newborns were born to O Rh D positive mothers, while 7.7% (1/13) were born to O Rh D negative mothers. The baseline immunohematological data of the 213 O group mothers and 13 newborns with ABO-HDN are described in Table 2.

3.3. Maternal IgG ABO antibodies in study population

The distribution of Anti-A/B antibodies was analysed in the O group mother. There was a significant difference between the group that developed ABO-HDN and the group that didn't develop ABO-HDN in terms of IgG Anti-A titer (W = 2396.500, p = <0.001) and IgG Anti-B titer (W = 2308.500, p = <0.001). The sensitivity and specificity of IgG Anti-A and IgG Anti-B titer for predicting ABO-HDN is illustrated in Fig. 1 in the form of a Receiver operating characteristic (ROC) curve. The area under the ROC curve (AUROC) for IgG Anti-A and IgG Anti-B for predicting ABO-HDN was 0.922 (95% CI: 0.815 - 1) and 0.888 (95% CI: 0.77 - 1), respectively thus demonstrating excellent diagnostic performance. It was statistically significant in both cases (p = <0.001).

3.4. Hemolysin test in high titre mothers (Titre≥256)

Of the 53 O group mothers with high ABO titer (≥256), 88.67% (n = 47) were hemolysin positive and 11.32% (n = 6) were hemolysin negative. Of the 47 hemolysin-positive mothers, ABO-HDN was present in 23.4% (n = 11) of newborns. There was no significant difference between the group that developed ABO-HDN (n = 11) and the group that didn't develop ABO-HDN (n = 2) in terms of hemolysin positivity. (X^2 =0.032, p = 1.000). α + β hemolysin was present in 58.4%(31/53), α hemolysin was present in 18.8%(10/53)) and β hemolysin was present

Table 2
Overview of the various immunohematological tests conducted on O group mothers and the newborns born to them (n = 213).

		ABO-HDN present (n = 13)	ABO-HDN absent (n = 200)	Statistical significance
IgM Anti A Median (IQR) (Range)	-	256 (256-512) (128 – 512)	32 (16-64) (4 – 512)	W= 2534 P value < 0.001
Median IgM Anti B (IQR) (Range)	-	256 (256-512) (64 – 1024)	32 (16-64) (4 – 512)	W= 2458.500 P value < 0.001
Median IgG Anti A (IQR) (Range)	-	512 (256-1024) (256 – 1024)	64 (32-128) (8 – 1024)	W= 2308.500 P value < 0.001
Median IgG Anti B (IQR) (Range)	-	512 (512-1024) (512 – 2048)	64 (32-128) (8 – 1024)	W= 2534 P value< 0.001
Hemolysin test*	Positive	11 (84.6%)	36 (90%)	$X^2 = 0.032$
	Negative	2 (15.1%)	4 (10%)	P value-1
DAT (by CAT)	Positive	12 (92.3%)	0 (0%)	$X^2 = 195.637$
	Negative	1 (7.69%)	200 (100%)	P value< 0.001
DAT (by CTT)	Positive	6 (46.7%)	0 (0%)	-
	Negative	7 (53.8%)	200 (100%)	
Elution studies	Positive	Anti A= 4 (44.4%)	-	-
		Anti B= 5 (55.5%)	-	-
	Negative	4	-	-

* For hemolysin positivity, ABO HDN negative cases (n = 40) were different, as the hemolysin test was performed in 53 high titre O group mothers.

in 11.32%(6/53) O group mothers.

3.5. DAT in newborns born to O group mothers as per institution protocol

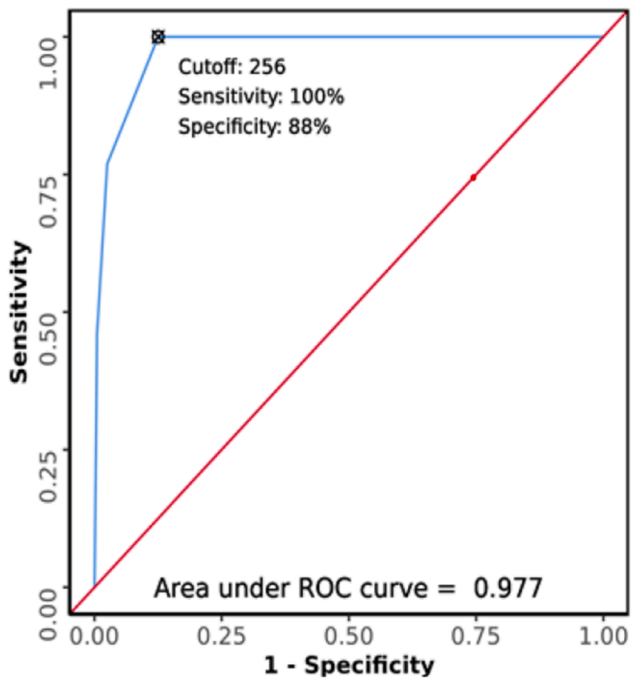
9.8% of ABO-incompatible newborns presented with positive DAT results. DAT was positive in 12 cases of ABO-HDN but negative in 1 case of ABO-HDN. There was a significant difference between the DAT positive and DAT negative groups in the distribution of ABO-HDN ($\chi 2 = 195.635$, p = <0.001). All participants (12/12) with positive DAT had ABO-HDN, and 0.9% (1/110) of the participants with negative DAT had ABO-HDN. Out of 12 DAT-positive cases, 58.3% had a titer of 1024, 16.64% had a titer of 512, 2048 each, and 8.32% had a titer of 256. The DAT was positive in 4.9% and 9.8% of ABO-incompatible newborns by CTT and CAT respectively. The positive predictive value of DAT was 100% by CAT and CTT, and the sensitivity by CAT was 92.3%, whereas, with CTT, it was 46%.

3.6. Elution studies

Heat elution was performed in all 13 cases of ABO-HDN, and 69.2% (9/13) were positive for specific anti-A/anti-B antibodies. Among the DAT-positive cases of ABO-HDN (n = 12), the eluate was positive for anti-A (n = 3) or anti-B (n = 5) in 66.67% (8/12) neonates. Eluate was also positive (for anti-A) for one case of DAT-negative ABO-HDN.

3.7. Diagnostic performance of different parameters in predicting ABO-HDN

The various immunohematological and clinical parameters were analyzed to determine their diagnostic performance in predicting the risk of ABO-HDN in the O group mothers, as shown in Table 3. The above tests were then ranked individually for different diagnostic parameters like sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Diagnostic accuracy as in Table 4. As per Table 3, maternal IgG titers had the highest Sensitivity & NPV values while DAT had the highest specificity & PPV values. Combining all the immunohaematological parameters, the diagnostic accuracy was 44%



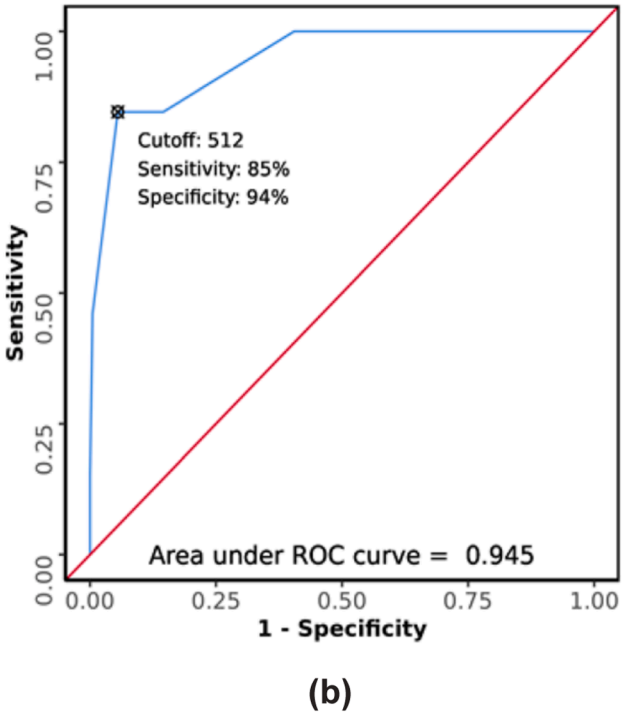


Fig. 1. (a,b): ROC Curve analysis showing diagnostic performance of IgG Anti-A & IgG Anti-B in Predicting ABO-HDN (n = 56, n = 66, respectively).

[95% CI, 39.2%– 50.1%] The combination of laboratory and clinical parameters improved the diagnostic accuracy to 52% [95% CI, 45.18%– 58.9%].

The cut-off value for anti-A & anti-B antibodies was defined as the value with the maximization of the Yuden index. The Yuden index was calculated at 87.5 and 79.1 for IgG Anti-A> 256 and IgG Anti-B> 512, respectively. The correlation between maternal IgG Ant-A/B and Total Bilirubin in ABO incompatible neonates is described in Fig. 2(a,b). For

Table 3
Performance of different study parameters in predicting ABO-HDN.

Variable	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
IgG Anti-A (Cutoff: 256)	100.0% (75-100)	87.5% (82-92)	34.2% (20-51)	100.0% (98-100)	88.3% (83-92)
IgG Anti-B (Cutoff: 512)	84.6% (55-98)	94.5% (90-97)	50.0% (28-72)	99.0% (96-100)	93.9% (90-97)
DAT	92.3% (64-100)	98.5% (96-100)	80.0% (52-96)	99.5% (97-100)	98.1% (95-99)
Hemolysin Test	84.6% (55-98)	13.3% (4-31)	29.7% (16-47)	66.7% (22-96)	34.9% (21-51)
Anemia	69.2% (39-91)	76.0% (69-82)	15.8% (7-28)	97.4% (94-99)	75.6% (69-81)
Hyperbilirubinemia	76.9% 46-95)	73.5% (67-79)	15.9% (8- 27)	98.0% (94-100)	73.7% (67-79)

Table 4
Ranking of different Diagnostic Parameters in predicting ABO-HDN.

Variable	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
IgG Anti-A (Cutoff: 256 by ROC)	1	3	3	1	3
IgG Anti-B (Cutoff: 512 by ROC)	3	2	2	3	2
DAT	2	1	1	2	1
Hemolysin Test	4	6	4	6	6
Anemia	6	4	6	5	4
Hyperbilirubinemia	5	5	5	4	5

every one-unit increase in Total Serum Bilirubin, the IgG Anti-A increased by 12.68 units (rho= 0.22, $p = 0.001$) while IgG Anti-B increased by 15.76 units, in ABO-incompatible newborns. [(rho= 0.18, $p = 0.009$)] A cut-off of IgG Anti-A ≥ 256 predicted the development of ABO-HDN in O group mothers with a sensitivity of 100% and a specificity of 88%. In comparison, a cut-off of IgG Anti-B ≥ 512 predicted the development of ABO-HDN with a sensitivity of 85% and a specificity of 94%.

The odds ratio (95% CI) of delivering newborns with A blood group who could have developed ABO-HDN ($n = 13$) when maternal IgG Anti-A ≥ 256 ($n = 38$) was 130 (27.15–622.42). Similarly, the odds ratio (95% CI) of delivering newborns with B blood group who could have developed ABO-HDN ($n = 13$) when maternal IgG Anti-B$\geq$ 512 ($n = 22$) was 170.57 (18.03–1613.77). The relative risk (95% CI) of delivering newborns with ABO-HDN when maternal IgG Anti-A ≥ 256 was 44 (14.23–134.65 while that when maternal IgG Anti-B $\geq$512 was 25.22 (10.7–53.74). However, there was no significant difference in the diagnostic performance of IgG Anti-A and IgG Anti-B (DeLong's Test $p = 0.126$).

3.8. Neonatal outcome

It is significant to note that out of 44 high titre (≥ 256) O group mothers with A/B group newborns, 29.5% (13/44) developed ABO-HDN while 43.2% (19/44) developed hyperbilirubinemia. Among neonates who developed ABO-HDN, 30.7% were mild cases while 69.2% were moderate cases. No severe ABO-HDN case was reported in our study. 69.2% cases of ABO-HDN cases received phototherapy for a duration ranging from 8.59 h to 36.6 h with a mean duration of 22.6 ± 14.01 h. 100% of the cases survived and were discharged. The hospital stay duration (Mean $\pm$ Standard Deviation) for the newborns with ABO-HDN was 79.7 ± 7.2 h.

4. Discussion

Based on the various cases of severe ABO-HDN reported in the literature from different parts of India or world, it has become imperative to study and estimate the risk involved in curbing the preventable ABO-HDN burden in the day-to-day healthcare delivery practices. It was estimated that 14.3% of deliveries in this part of Northern India would result in O blood group mothers giving birth to a child with a non-group O phenotype. In the present study, 18% of group O mothers had IgG anti A/B > 512. Considering this, about 2.57% of institutional deliveries in our center were at risk of ABO-HDN, hence anticipating therapeutic intervention. Against this predictive data, the present study has shown a disease prevalence of 10.6% in O group mothers ABO incompatible with newborns, 6.1% in O group mothers, and 1.7% in total deliveries conducted during the study period. Similar studies have been done by Akanmu et al. in Africa and Patale et al. in North India, where they calculated a disease prevalence of 4.3% and 2.96%, respectively and suggested neonatal jaundice due to ABO-HDN as an essential cause of neonatal morbidity [5,9].

The potential of antenatal screening of O group mothers at risk of ABO-HDN was investigated by IgG ABO titer and hemolysin positivity. A positive relationship was observed between maternal IgG antibody titer levels and disease prevalence. The IgG titer levels in mothers with ABO-HDN newborns ranged from 256 to 2048, which may vary based on study population characteristics [10,11]. A linear association was noted between maternal IgG Anti A/B and Total Bilirubin in ABO-incompatible newborns [(rho= 0.22, $p = 0.001$); (rho= 0.18, $p = 0.009$)] thereby substantiating the need of screening for clinically significant ABO antibodies in O group mothers. All the mothers who delivered newborns with ABO-HDN had IgG titer$\geq$ 256. (p-value <0.01). A high IgG titer level ≥ 512 was significantly associated with ABO-HDN (p-value <0.001), which is in accordance with studies conducted all over the world [12–15]. In the current study, a titer value of ≥ 256 and ≥ 512 was considered as high titer for IgG Anti A and IgG Anti B respectively, calculated based on the maximization of the Yuden index. However, critical titer values and the predictability of titer levels remain controversial, depending on an array of factors such as geography, race, testing platform, and timing of testing, among others. In our study population, ABO titration showed a sensitivity of 84 to 100% and a specificity of 87.5 to 94.5% for the prediction of ABO-HDN. In the present study population with 57% ABO incompatibility and 10.6% disease prevalence, this may help in predicting the high-risk pregnancies that may warrant extra neonatal care and support. 29.5% of O group mothers with high ABO titre developed newborns with ABO HDN while 43.2% of them developed newborns with hyperbilirubinemia. In this study, as per institution protocol, the newborns with hyperbilirubinemia were followed up for the duration of hospital stay and discharged after two consecutive Total Bilirubin was within the normal range. It is suggested that newborns born to mothers with high ABO titers should be followed up for a longer duration to monitor for the signs of jaundice, especially following early discharge after delivery. In our study, maternal titrations were done as per inclusion criteria when the patients got admitted at the time of delivery because maternal antibody titers are the highest in the third trimester [15]. The Positive Predictive value of IgG Anti A was 32.4%, while that of IgG Anti B was 47.6% which was relatively low for routine screening. However, given the low cost of reagent Dithiothreitol and the non-cumbersome routine titration technique, this may be

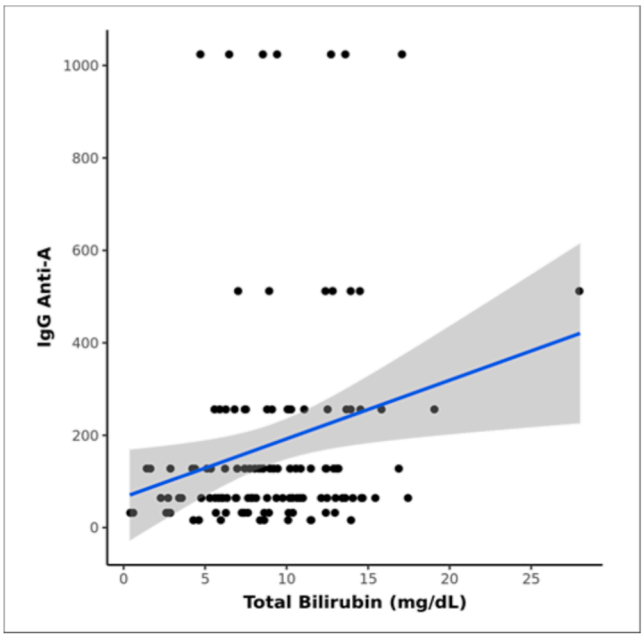


Fig. 2. (a,b): Scatter plot diagram showing the correlation between Total Bilirubin (mg/dL) and IgG Anti-A/Anti-B in ABO Incompatible newborns (n = 122).

introduced as part of routine antenatal care. At the same time, further research and development may continue to improve the predictive value.

The mothers with high IgG ABO titers (≥256) were assessed for hemolysin positivity and a strong correlation was demonstrated between high IgG titers and strong hemolysin positivity. There was no significant difference between hemolysin-positive and negative mothers in terms of the development of ABO-HDN ($\chi 2 = 0.032$, $p = 1.000$). Similar studies were conducted by Mathai et al. and Bastos et al., where they used the hemolysin test as a screening test to identify donors with high IgG titers [16–18]. In this study, maternal titer> 256 was adopted as a critical titer since isoagglutinin titers keep rising throughout the length of pregnancy due to trans-placental transfer and fetal ABO antigen stimulation. The hemolytic activity of IgG depends on the IgG subclass implicated, which can explain the 11.32% high-titer mothers who showed negative or weak hemolysin grades despite having high IgG titers. With a sensitivity of 84.6%, specificity of 13.3%, PPV of 29.7%, and NPV of 66.7%, it remains a question mark whether the hemolysin test can be used as a stand-alone test. However, it may be used in conjunction with other immunohematological tests.

In the present study, the prevalence of DAT positivity in the ABO-incompatible newborn was 9.8%. The prevalence of DAT positivity in the different study populations across the world is shown in Table 5. The difference in DAT positivity is due to various reasons such as the study population characteristics like a population with high IgG titers or the testing platform and related sensitivity [15,19–22]. DAT was performed by both CAT and CTT methods for comparison to predict the occurrence of ABO-HDN by both techniques. CAT was found to be a more sensitive technique of determining DAT positivity. Dittmar et al. suggested that if DAT is negative by CTT, assays with increased Sensitivity, like CAT or Flow cytometry, may be preferred for detecting low levels of red cell-bound or low-affinity IgG antibodies [23]. In the present study, 50% of positive DAT results showed relatively weaker strength (strength≤2). This study also reported one case of DAT-negative ABO-HDN. This discrepancy may be because the A and B antigenic sites are weakly expressed on neonatal RBC membranes and can attach very little anti-A or anti-B, which may limit the sensitivity of the DAT [3].

The current study used the heat elution technique for all the cases of ABO-HDN. One DAT-negative case of ABO-HDN was diagnosed based on clinical suspicion, and that eluate was positive for the maternal anti-A antibody. Elution studies carry special diagnostic value in settings of DAT-negative ABO-incompatible newborns with high clinical suspicion of ABO-HDN. The last wash was negative as per the quality assurance of the procedure performed. Routinely performing elution may have a little advantage as previous investigators have shown that it is not uncommon to get cord blood eluates positive for A or B antigens when there is an ABO incompatibility between the mother and her child, even when DAT is negative [27].

Our study aimed to investigate the usefulness of different laboratory and clinical parameters tested on mother's sample and cord blood, both in isolation and combined, for early diagnosis of ABO-HDN. Although the specificity of the immunohematological tests like maternal IgG titers and newborn DAT positivity is high, their ability to individually predict the disease's severity is poor. [29] Based on the findings of this study, combining both immunohematological and clinical parameters can improve the accuracy of diagnosing a case of ABO-HDN. Hence, a holistic multidisciplinary approach by integrating the immunohematological tests, clinical symptoms, and hematological parameters can identify newborns at risk who require active intervention.

The current study was conducted during the peak COVID-19 pandemic in India and the actual prevalence could be different in normal times. The sample size of our study was comparatively smaller than other studies conducted. Also, the study was limited to O-group mothers, so a comprehensive risk assessment for the disease entity was

Table 5
Prevalence of DAT positivity in the different study populations in studies conducted across the world.

Authors	Place of study	Year of study	DAT positivity
Present study	**North India**	**2021**	**9.8%**
Das S et al.[15]	South India	2021	11.2%
Bhatt et al.[24]	South India	2016	1.9%
Valsami et al.[25]	Greece	2014	2.5%
Procianoy et al.[19]	Italy	1987	4.5%
Comos JB et al.[20]	Spain	1991	2.9%
Quinn et al.[21]	United Kingdom	1988	15.6%
Toy et al.[26]	USA	1988	39.5%

not possible.

However, a similar multicentric study can be planned for an overall risk assessment of ABO-HDN in different parts of the country. Educational programs may be conducted to create awareness among the treating obstetricians and neonatologists for active management of O group pregnancies with high maternal titer. Active antenatal screening may be recommended as routine investigations in all O group mothers. A more extended hospital stay may be necessary to implement close follow-up of the high-risk newborns.

5. Conclusion

Mothers with IgG antibody titer$\geq$ 256 should be followed up for the development of ABO-HDN. Titre $\geq$ 512 strongly correlates with features of ABO-HDN. DTT treatment for IgG Anti-A/B titration can be performed as a routine test in O-group mothers which involves no significant expenditure and can help identify high-risk mothers. Maternal IgG titers have the highest sensitivity & NPV, while DAT has the highest specificity & PPV. Both tests can be performed for an early diagnosis of ABO-HDN. The high-risk newborns should be monitored for signs and symptoms of anemia and hyperbilirubinemia so that an early diagnosis can be made and appropriate intervention can be taken in due time to avoid serious complications. Institutional delivery for all high-risk cases and dedicated multi-disciplinary follow-up of newborns with ABO-HDN is advocated.

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CRediT authorship contribution statement

Joyisa Deb: Conceptualisation, Methodology, Analysis, Investigation, Data curation, Writing – original draft, Project administration, Visualisation. **Ashish Jain**: Conceptualisation, Analysis, Validation, Resources, Writing – review & editing, Visualisation, Supervision. **Daljit Kaur**: Conceptualisation, Resources, Writing – review & editing, Validation, Supervision. **Anupama Bahadur**: Conceptualisation, Resources, Writing – review & editing, Supervision. **Sriparna Basu**: Conceptualisation, Resources, Writing – review & editing, Supervision. **Gita Negi:** Conceptualisation, Resources, Writing – review & editing, Validation, Supervision.

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None.

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